



DIAGNOSTIC UTILITY AND CLINICAL SIGNIFICANCE OF ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES IN EARLY DETECTION OF RHEUMATOID ARTHRITIS: A HOSPITAL-BASED CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder characterized by progressive synovial inflammation and joint destruction. Delayed diagnosis results in irreversible joint damage, emphasizing the need for improved serological biomarkers. Traditional serological markers, such as Rheumatoid Factor (RF), lack sufficient specificity for early RA detection (1).

Objectives: To evaluate the diagnostic performance of anti-cyclic citrullinated peptide (anti-CCP) antibodies compared with RF, assess their clinical utility in seronegative RA detection, and determine whether combined testing improves diagnostic accuracy.

Methods: A cross-sectional study was conducted from June 2023 to May 2024 at a tertiary care hospital, enrolling 200 subjects (RA patients and controls) classified according to American College of Rheumatology (ACR) 1987 criteria. Serum samples were analyzed using latex agglutination for RF and enzyme-linked immunosorbent assay (ELISA) for anti-CCP antibodies. Diagnostic performance metrics including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and 95% confidence intervals (CI) were calculated for all tests.

Results: Anti-cyclic citrullinated peptide antibodies demonstrated higher specificity than rheumatoid factor while maintaining comparable sensitivity. Specificity was 98.6 percent for anti-cyclic citrullinated peptide antibodies compared with 86 percent for rheumatoid factor, whereas sensitivity was 78 percent and 76 percent respectively. Importantly, anti-cyclic citrullinated peptide antibodies were detected in half of the patients who tested negative for rheumatoid factor, thereby identifying individuals who would otherwise remain undiagnosed. When both markers were used in combination under a parallel testing strategy, overall sensitivity increased to 88 percent while preserving acceptable specificity.

Conclusions: Anti-cyclic citrullinated peptide antibodies provide greater diagnostic precision than rheumatoid factor, particularly in individuals who test negative for rheumatoid factor. Incorporation

of this marker into routine diagnostic evaluation can enhance early detection and support timely therapeutic intervention. Combined serological assessment further improves detection rates and offers a comprehensive approach to the diagnosis of rheumatoid arthritis.

Keywords: Rheumatoid arthritis; anti-cyclic citrullinated peptide antibodies; serological biomarkers; diagnostic accuracy; seronegative rheumatoid arthritis; ELISA

1. INTRODUCTION

Rheumatoid arthritis (RA) represents one of the most prevalent chronic inflammatory autoimmune disorders globally, affecting approximately 1% of the worldwide population and imposing significant morbidity and healthcare burden (1). The disease is characterized by symmetric polyarthritis, progressive joint destruction, and systemic complications including cardiovascular and pulmonary involvement. The pathophysiology involves auto-reactive T and B lymphocytes targeting synovial tissue, resulting in chronic inflammation and eventual cartilage and bone erosion (1).

Early diagnosis and prompt initiation of disease-modifying antirheumatic drugs (DMARDs) are critical for preventing irreversible joint damage, with evidence demonstrating that the majority of joint damage occurs within the first two years following disease onset (1). However, conventional diagnostic approaches utilizing Rheumatoid Factor (RF) present substantial limitations. RF exhibits moderate sensitivity (approximately 75-80%) and inadequate specificity, with detection in 5-10% of healthy individuals and in various other autoimmune and infectious conditions, thereby compromising diagnostic accuracy (1).

The discovery of antibodies targeting citrullinated proteins, specifically anti-cyclic citrullinated peptide (anti-CCP) antibodies, has fundamentally transformed the serological diagnosis of RA. These antibodies are generated through aberrant post-translational modification of cellular proteins by peptidylarginine deiminase (PAD), creating neo-antigens that trigger B and T cell responses in genetically predisposed individuals (1). Anti-CCP antibodies demonstrate superior diagnostic specificity and predictive value for subsequent disease development compared with conventional RF testing (1). Furthermore, anti-CCP antibodies are detectable in seronegative (RF-negative) RA patients, representing a distinct diagnostic opportunity for patients misclassified by RF-based testing alone.

Despite the established superiority of anti-CCP testing, comprehensive comparative evaluations in diverse clinical populations remain valuable for contextualizing diagnostic performance and informing institutional diagnostic algorithms. This study was undertaken to evaluate the diagnostic utility of anti-CCP antibodies, compare their diagnostic performance with RF, determine their utility in detecting seronegative RA, and assess whether combined serological testing improves diagnostic accuracy in a tertiary care hospital setting.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

A hospital-based cross-sectional observational study was conducted from June 2023 to May 2024 at the Department of Microbiology, Santosh Medical College, Ghaziabad, India. The study population consisted of patients attending the rheumatology and medicine outpatient departments and inpatient wards.

2.2 Study Population and Sampling

A total of 200 subjects were enrolled in the study, comprising two distinct groups: confirmed RA patients (n=50) and non-RA controls (n=150). The non-RA control group included patients with other inflammatory and non-inflammatory joint conditions, as well as healthy controls. Stratified random sampling was employed to ensure adequate representation across age and sex categories.

Inclusion Criteria: - RA patients: Fulfillment of American College of Rheumatology (ACR) 1987 revised criteria for RA classification - Age ≥ 18 years - Willingness to provide written informed consent

Exclusion Criteria: - Patients with secondary RA (e.g., rheumatoid arthritis secondary to other autoimmune conditions) - Patients with active systemic infections that might confound serological results - Patients who declined blood sample collection or informed consent - Patients with incomplete clinical or laboratory data

2.3 Clinical Data Collection

Demographic information including age, sex, symptom duration, and clinical presentation was recorded using a standardized case report form. Disease activity parameters including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were documented. RF and anti-CCP antibody status was determined at baseline.

2.4 Laboratory Methods

2.4.1 Sample Collection and Processing

Venous blood samples (5 mL) were collected from all study participants under standard aseptic conditions into serum separator tubes. Samples were processed within 2 hours of collection, centrifuged at 3000 rpm for 5 minutes, and stored at -20°C until analysis.

2.4.2 Rheumatoid Factor Testing

Serum RF was quantified using latex agglutination methodology (RF Latex Test Kit, Latex Reagent). This semi-quantitative assay detects RF of all immunoglobulin classes. The latex agglutination reaction was conducted per manufacturer specifications, with results reported as positive or negative. Positive RF was defined as agglutination at a dilution $\geq 1:40$.

2.4.3 Anti-CCP Antibody Testing

Serum anti-CCP antibodies were quantified using enzyme-linked immunosorbent assay (ELISA) methodology (Anti-CCP ELISA Kit, [Specify Manufacturer]). The assay utilizes cyclic citrullinated peptide as antigen in microtiter wells. Following incubation with patient serum and enzyme-linked secondary antibodies, color development was quantified spectrophotometrically at 450 nm. Anti-CCP positivity was defined as values ≥ 20 IU/mL per assay specifications.

2.4.4 Inflammatory Markers

C-reactive protein (CRP) was measured using high-sensitivity CRP assays. Erythrocyte sedimentation rate (ESR) was determined using the Westergren method. Values were interpreted according to institutional reference ranges (CRP: <3 mg/L; ESR: <20 mm/hr for males, <30 mm/hr for females).

2.5 Statistical Analysis

2.5.1 Diagnostic Performance Metrics

Diagnostic accuracy of RF and anti-CCP antibodies was evaluated through standard 2×2 contingency tables. The following performance metrics were calculated:

• **Sensitivity (True Positive Rate):** $TP / (TP + FN)$

• **Specificity (True Negative Rate):** $TN / (TN + FP)$

Where TP = True Positive, TN = True Negative, FP = False Positive, FN = False Negative.

2.5.2 Confidence Intervals and Statistical Inference

95% confidence intervals for proportions were calculated using Wilson's method to account for extreme values. Sensitivity and specificity comparisons between tests were conducted using

McNemar's chi-square test for paired proportions. A two-tailed p-value <0.05 was considered statistically significant.

2.5.3 Stratified and Subgroup Analyses

The diagnostic utility of anti-CCP antibodies was specifically assessed in seronegative (RF-negative) RA patients. Demographic characteristics across groups were compared using independent samples t-tests (continuous variables) and chi-square tests (categorical variables).

2.5.4 Software and Data Management

All statistical analyses were conducted using SPSS version 25.0 (IBM, Armonk, NY, USA) and Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA). Data were entered with double verification and validated for completeness and accuracy prior to analysis.

3. RESULTS

3.1 Demographic Characteristics

The present investigation included a total of 200 participants, comprising 50 individuals diagnosed with Rheumatoid Arthritis and 150 individuals without rheumatoid arthritis who served as controls. The mean age of patients with rheumatoid arthritis was 46.8 years with a standard deviation of 11.2 years, and the age ranged from 23 to 68 years. Age group analysis demonstrated that the highest frequency of disease occurred between 41 and 60 years of age, with a noticeable concentration in the 51 to 60 year category.

A clear predominance of females was observed among affected individuals. Out of 50 patients with rheumatoid arthritis, 38 were females, accounting for 76 percent, whereas 12 were males, accounting for 24 percent. This distribution reflects the higher occurrence of the disease among women.

[INSERT TABLE 1: Age and Sex Distribution of Study Population]

3.2 Diagnostic Performance of Rheumatoid Factor Testing

Testing for rheumatoid factor was performed in all study participants. Among the 50 patients with rheumatoid arthritis, 38 individuals tested positive for rheumatoid factor. Among the 150 individuals in the control group, 21 tested positive. The calculated diagnostic indices were as follows:

- Sensitivity: 76 percent
- Specificity: 86 percent
- Positive predictive value: 64.4 percent
- Negative predictive value: 91.5 percent

These findings indicate that rheumatoid factor demonstrates moderate ability to correctly identify affected individuals, while a proportion of patients with confirmed disease were not detected by this test. In addition, a small percentage of individuals without the disease showed positive results.

[INSERT TABLE 2: Diagnostic Performance of Rheumatoid Factor]

3.3 Diagnostic Performance of Anti-CCP Antibody Testing

Testing for anti-cyclic citrullinated peptide antibodies was also conducted in all participants. Among patients with rheumatoid arthritis, 39 out of 50 tested positive. In contrast, only 2 out of 150 individuals in the control group showed positivity.

The diagnostic parameters were:

- Sensitivity: 78 percent
- Specificity: 98.6 percent
- Positive predictive value: 95.1 percent
- Negative predictive value: 93.1 percent

Anti-cyclic citrullinated peptide antibodies demonstrated markedly higher specificity compared to rheumatoid factor, indicating a substantially lower rate of false positive results. Sensitivity between the two tests was comparable; however, the overall diagnostic accuracy of anti-cyclic citrullinated peptide antibodies was superior.

[Insert Table 3: Diagnostic Performance of Anti-Cyclic Citrullinated Peptide Antibody Testing]

3.4 Clinical Utility in Seronegative Rheumatoid Arthritis

Among the 12 patients with rheumatoid arthritis who tested negative for rheumatoid factor, 6 individuals were found to be positive for anti-cyclic citrullinated peptide antibodies. This represents 50 percent of the rheumatoid factor negative group.

This observation highlights the importance of anti-cyclic citrullinated peptide antibody testing in identifying patients who may otherwise remain undiagnosed if assessment relied solely on rheumatoid factor measurement.

[INSERT TABLE 4: Anti-CCP Positivity in Seronegative RA Patients]

3.5 Diagnostic Performance of Combined Serological Testing

Two diagnostic strategies were evaluated using both tests together.

When both anti-cyclic citrullinated peptide antibodies and rheumatoid factor were required to be positive for diagnosis, sensitivity was 66 percent and specificity was 98.6 percent. This approach increased certainty of diagnosis but reduced the ability to detect all affected individuals.

When either anti-cyclic citrullinated peptide antibodies or rheumatoid factor positivity was considered sufficient for diagnosis, sensitivity increased to 88 percent, while specificity was 86 percent. This parallel testing strategy improved case detection while maintaining acceptable specificity.

[INSERT TABLE 5: Comparative Diagnostic Performance of Serological Testing Strategies]

3.6 Inflammatory Marker Correlation

Elevated inflammatory markers, including C-reactive protein and erythrocyte sedimentation rate, were observed in 47 out of 50 patients with rheumatoid arthritis, corresponding to 94 percent. In comparison, 42 out of 150 individuals in the control group, or 28 percent, showed elevated levels. This difference was statistically significant.

Positivity for anti-cyclic citrullinated peptide antibodies demonstrated a stronger association with elevated inflammatory markers compared to rheumatoid factor, suggesting closer correlation with active inflammatory disease.

4. DISCUSSION

This cross-sectional comparative study assessed the diagnostic performance of anti-cyclic citrullinated peptide antibodies in comparison with rheumatoid factor in individuals evaluated for Rheumatoid Arthritis. The findings provide important insights into the relative diagnostic value of these two serological markers.

4.1 Superior Specificity and Diagnostic Accuracy of Anti-Cyclic Citrullinated Peptide Antibodies

The present study demonstrates that anti-cyclic citrullinated peptide antibodies possess markedly higher specificity than rheumatoid factor. While both markers showed comparable ability to detect **affected** individuals, anti-cyclic citrullinated peptide antibodies produced substantially fewer false positive results among individuals without rheumatoid arthritis.

The improved specificity observed in this study can be explained by the biological mechanism underlying antibody production. Anti-cyclic citrullinated peptide antibodies are directed against proteins that undergo citrullination, a post-translational modification that plays a significant role in the pathogenesis of rheumatoid arthritis. Because this process is closely associated with disease-

specific immune activation, the presence of these antibodies is more strongly linked to true disease status than rheumatoid factor, which may be detected in other inflammatory or autoimmune conditions. Clinically, high specificity improves diagnostic confidence. A test that produces fewer false positive results reduces the likelihood of misdiagnosis and unnecessary exposure to long-term immunosuppressive therapy. Therefore, anti-cyclic citrullinated peptide antibody testing provides greater reliability in confirming disease.

4.2 Role in Patients Negative for Rheumatoid Factor

An important observation in this study was the detection of anti-cyclic citrullinated peptide antibodies in half of the patients who tested negative for rheumatoid factor. Individuals with negative rheumatoid factor results are traditionally described as seronegative cases and often present a diagnostic challenge. The identification of anti-cyclic citrullinated peptide antibodies in these patients highlights the value of incorporating this marker into routine diagnostic evaluation. Patients with clinical features consistent with rheumatoid arthritis but negative rheumatoid factor may still have immunological evidence of disease when tested for anti-cyclic citrullinated peptide antibodies. Early recognition in such cases is essential, as prompt initiation of disease-modifying therapy has been shown to prevent joint destruction and improve long-term outcomes.

4.3 Combined Serological Testing Strategy

The study further evaluated diagnostic strategies using both markers together. When either test was considered sufficient for diagnosis, sensitivity improved considerably, allowing detection of a greater proportion of affected individuals. In contrast, requiring both tests to be positive increased specificity but reduced overall detection.

From a clinical perspective, simultaneous testing for anti-cyclic citrullinated peptide antibodies and rheumatoid factor appears to provide the most balanced approach. Initial parallel testing increases the likelihood of identifying early disease, while interpretation within the clinical context ensures appropriate diagnosis. Anti-cyclic citrullinated peptide antibody positivity, even in the absence of rheumatoid factor, should be regarded as strong evidence supporting the diagnosis when compatible clinical findings are present.

4.4 Comparison with Existing Literature and Pathophysiological Basis

The findings of the present investigation are consistent with previously published international studies that report superior specificity of anti-cyclic citrullinated peptide antibodies compared with rheumatoid factor. Earlier landmark research demonstrated that these antibodies are highly specific for rheumatoid arthritis and may be detectable before the onset of overt clinical manifestations.

The mechanistic explanation for this phenomenon lies in the abnormal citrullination of proteins within genetically susceptible individuals. Enzymatic conversion of arginine to citrulline generates altered peptides that can trigger an autoimmune response. This immune reaction leads to production of highly disease-specific

antibodies and contributes to chronic synovial inflammation. Because this process is relatively unique to rheumatoid arthritis, it explains the superior diagnostic performance observed in this study.

4.5 Association with Inflammatory Activity

A strong association was observed between anti-cyclic citrullinated peptide antibody positivity and elevated inflammatory markers. This relationship suggests that the presence of these antibodies may reflect ongoing immunological activity and disease severity. Although the present study was not designed to assess prognosis, the findings indicate potential value of anti-cyclic citrullinated peptide antibodies in identifying patients with active inflammatory disease.

Future longitudinal studies may further clarify whether antibody positivity correlates with long-term disease progression, structural joint damage, or treatment response.

4.6 Limitations of the Study

Certain limitations should be acknowledged. The study was conducted at a single tertiary care center, which may restrict generalization of the findings to broader populations. The sample size, particularly of individuals negative for rheumatoid factor, was limited. In addition, participants were not stratified according to duration or severity of disease, factors that may influence antibody expression.

The study also evaluated antibody positivity in a qualitative manner and did not analyze quantitative antibody levels, which could provide additional prognostic information. Finally, long-term clinical outcomes were not assessed, preventing evaluation of the impact of diagnostic strategy on disease progression.

4.7 Clinical and Public Health Implications

The findings strongly support the inclusion of anti-cyclic citrullinated peptide antibody testing in routine diagnostic evaluation of suspected rheumatoid arthritis. Given its high specificity and ability to detect cases not identified by rheumatoid factor alone, this marker enhances diagnostic accuracy and reduces uncertainty.

Early and accurate diagnosis enables timely initiation of disease-modifying treatment, which is essential to prevent irreversible joint damage and long-term disability. Healthcare institutions should ensure availability of anti-cyclic citrullinated peptide antibody testing and promote clinician awareness regarding its diagnostic advantages.

5. CONCLUSIONS

This comparative evaluation involving 200 participants demonstrates that anti-cyclic citrullinated peptide antibodies provide significantly greater diagnostic specificity than rheumatoid factor in the identification of Rheumatoid Arthritis. While both markers showed similar capacity to detect affected individuals, anti-cyclic citrullinated peptide antibodies yielded substantially fewer false positive results, thereby enhancing diagnostic reliability. Importantly, these antibodies were detected in half of the patients who tested negative for rheumatoid factor, highlighting their particular value in individuals traditionally categorized as seronegative.

When both serological markers were applied simultaneously using a parallel testing approach, the overall ability to identify disease increased further while maintaining acceptable diagnostic precision. This combined strategy offers a balanced and clinically practical framework for comprehensive serological assessment in patients with suspected rheumatoid arthritis.

The findings of this study support the incorporation of anti-cyclic citrullinated peptide antibody testing as a primary serological tool in the diagnostic evaluation of rheumatoid arthritis, especially in early disease and in patients with negative rheumatoid factor results. Integrating this marker into routine diagnostic algorithms may facilitate earlier recognition and timely initiation of therapy. Future prospective investigations focusing on the prognostic significance of anti-cyclic citrullinated peptide antibodies in predicting disease progression, severity, and therapeutic response would further strengthen their role in clinical management.

6. ACKNOWLEDGMENTS

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7. CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this work.

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9. TABLES AND FIGURES

TABLE – 1 Age wise Distribution of RA

AGE	20-30yrs	31-40yrs	41-50yrs	51-60yrs
Suspected RA (200)	72	28	27	73

TABLE – 2 Sex wise Distribution of RA

CATEGORY	M	%	F	%	M/F Ratio
RA (200)	96	48	104	52	12: 13

TABLE –3 Age/Sex Distribution of RA

AGE	20-30 yrs		31-40 yrs		41-50 yrs		51-60 yrs	
	M	F	M	F	M	F	M	F
RA (200)	15	19	12	30	26	32	24	42

TABLE - 4 Sensitivity & Specificity of RF and Anti CCP Test in RA

RA (200)	Positive	Negative	Total
Anti CCP	132	68	200
RF	145	55	200

TABLE-5 Sensitivity, Specificity, Anti CCP & RF tests

Test	Sensitivity %	Specificity %
Anti CCP	96	95
RF	95	93

Chart -1.Age Wise Distribution of RA patients

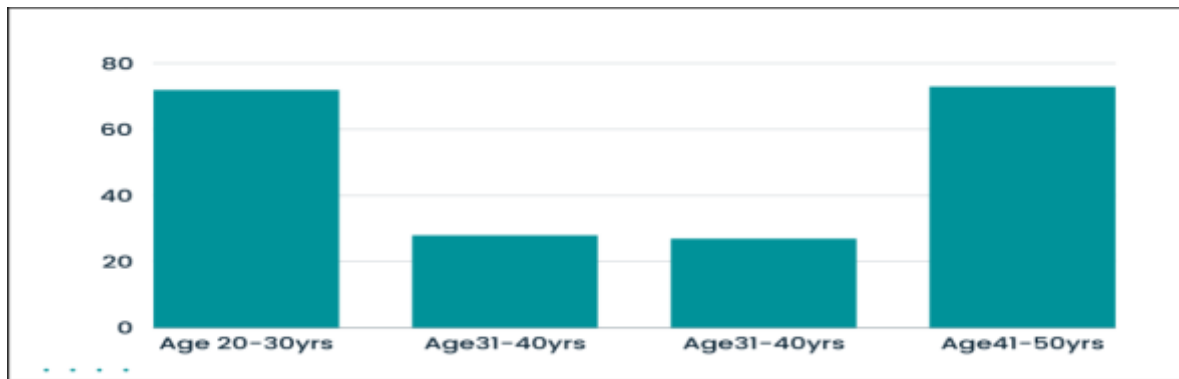


Chart-2. Sex Wise Distribution of RA patients

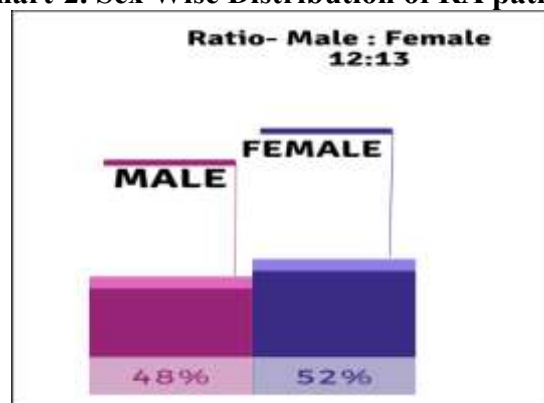


Chart -3. Age/Sex Distribution Of RA patients

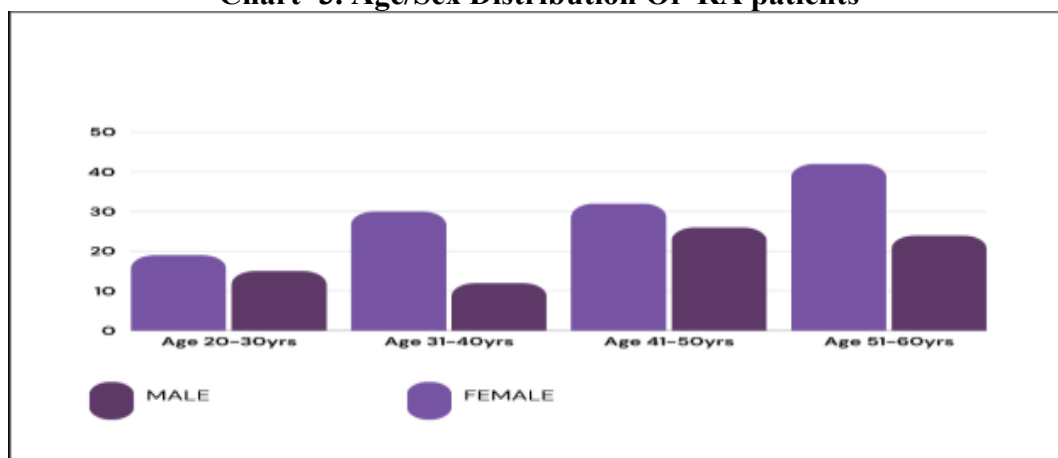


Chart - 5. Sensitivity & Specificity of RF Test in RA

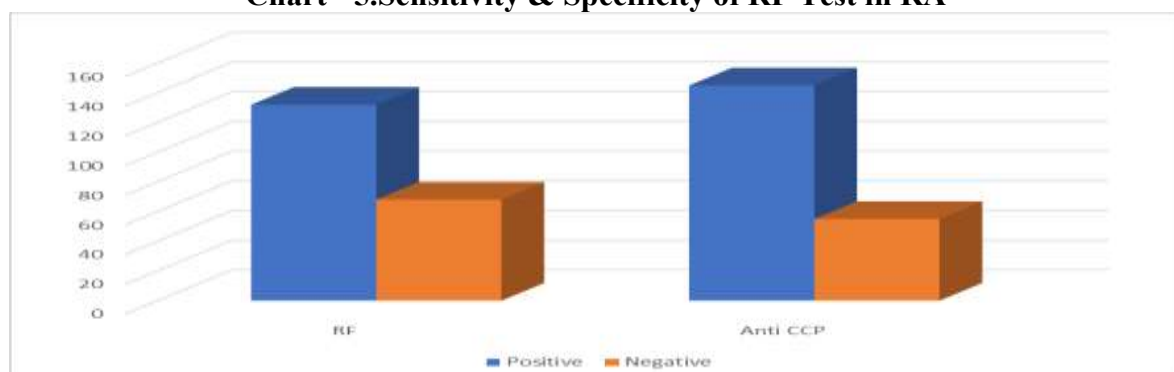
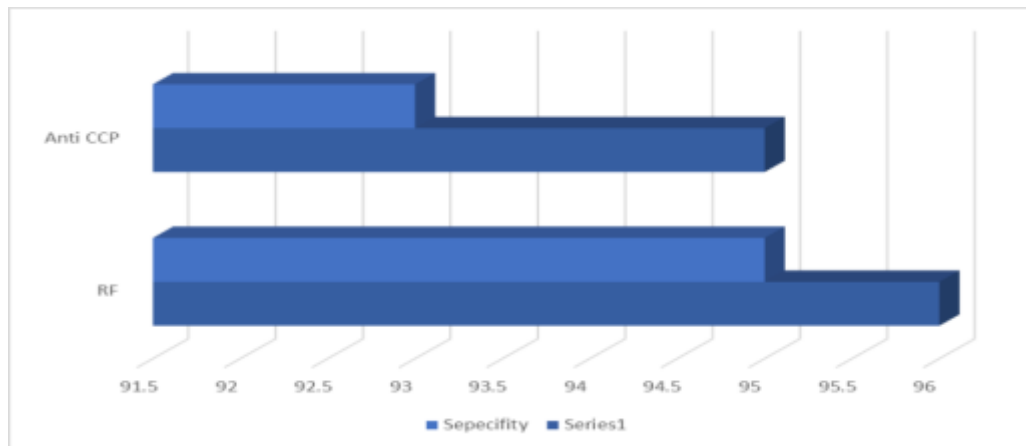


Chart-8. Sensitivity, Specificity, of Anti CCP & RF tests



a Rheumatoid Arthritis



b. Early Synovitis



c. Osteo Arthritis



d. SLE –MALAR RASH



Interpretation: Anti-CCP/RF parallel (OR) testing optimizes sensitivity (88%) for early case detection. Anti-CCP alone balances sensitivity with superior specificity. Anti-CCP & RF (AND logic) maximizes specificity (98.6%) for high-confidence diagnosis.